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Improved Method for Feeding and Preparation of Small-Volume Cell Cultures

JOHN W. KREIDER

Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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The use of Cornwall automatic pipettes (Becton, Dickinson and Co., Rutherford, N.J.) for the planting of replicate cell suspensions into multiple culture vessels is a routine procedure in some tissue-culture laboratories. This instrument withdraws samples from the bottom of an Erlenmeyer flask in which the cell suspension is continuously stirred by a Teflon-coated magnetic bar. The same apparatus is also valuable in the replacement of media in monolayer cultures.

One disadvantage of this pipette is that the design of the apparatus requires the introduction of the aspirating portion, a latex tubing terminating in a hollow metal sinker into the flask. Not only is the exterior of the tubing easily contaminated during transfer to the flask, but the sinker may periodically collide with the Teflon-coated magnetic bar rotating in the bottom of the flask. In addition, the passage of the latex tubing out of the neck of the flask requires a loose-fitting closure to eliminate kinking of the tube. This closure, sometimes composed of cheesecloth, cotton, or aluminum foil, is also vulnerable to airborne contamination.

Because of the difficulties listed above, a modification to the apparatus is proposed which minimizes the problems. These modifications are illustrated in Fig. 1. An Erlenmeyer flask (300 to 1,000 ml) was fitted with a serum bottle orifice with attached rubber cap and clinched-on metal skirt. The metal sinker of the Cornwall apparatus is replaced by a male Luer-Lok hose end (B-D, Co.; #H/468L). For the preparation of replicate cultures, the surface of the rubber cap is sterilized with 70% ethyl alcohol and flame and punctured with a no. 18 gauge disposable needle which has been attached to the Luer-Lok on the end of the latex tubing. The magnetic stirring bar and the cell suspension are then added to the flask by removing the screw cap. With media alone, the apparatus is convenient for routine feeding of cultures. An additional advantage is that media for several periodic feedings may be stored in the flask, eliminating the necessity of transfer. It is recommended that a single rubber cap be punctured no more than three times, since some leakage occurs with repeated injections. The cap and metal skirt should be removed for thorough cleaning and a new assembly replaced prior to autoclaving. Alternatively, the cap assembly may be replaced by a one-piece needle-puncture rubber stopper which has a sleeve-like extension that folds over the neck of the orifice (A. H. Thomas Co., Philadelphia, Pa.; #8826). This latter stopper is simpler to use but relatively expensive, and the thin wall does not permit the needle tip to be positioned at the bottom of the flask.

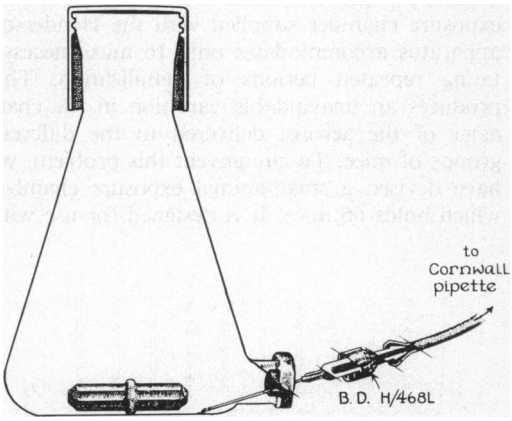

FIG. 1. *Modified Cornwall apparatus.*

In summary, the modification of the apparatus described here results in greater convenience and improved aseptic procedure in the preparation and feeding of small-volume tissue cultures.